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# The light-harvesting complexes of higher-plant Photosystem I: Lhca1/4 and Lhca2/3 form two red-emitting heterodimers

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The outer antenna of higher-plant PSI (Photosystem I) is composed of four complexes [Lhc (light-harvesting complex) a1–Lhca4] belonging to the light-harvesting protein family. Difficulties in their purification have so far prevented the determination of their properties and most of the knowledge about Lhcas has been obtained from the study of the *in vitro* reconstituted antennas. In the present study we were able to purify the native complexes, showing that Lhca2/3 and Lhca1/4 form two functional heterodimers. Both dimers show red-fluorescence emission with maxima around 730 nm, as in the intact PSI complex. This indicates that the dimers are in their native state and that LHCI-680, which was previously assumed to be part of the PSI antenna, does

not represent the native state of the system. The data show that the light-harvesting properties of the two dimers are functionally identical, concerning absorption, long-wavelength emission and fluorescence quantum yield, whereas they differ in their high-light response. Implications of the present study for the understanding of the energy transfer process in PSI are discussed. Finally, the comparison of the properties of the native dimers with those of the reconstituted complexes demonstrates that all of the major properties of the Lhcas are reproduced in the *in vitro* systems.

**Key words:** antenna complex, light-harvesting complex (Lhc), light reaction, photoprotection, photosynthesis, Photosystem I.

## INTRODUCTION

In the first steps of photosynthesis, light energy is captured and converted into chemical energy. In higher plants and algae, this process takes place in the thylakoid membrane, where PSII (Photosystem II) and PSI work in concert with cytochrome *b<sub>6</sub>f* and ATP synthase to harvest the light and store its energy by generating ATP and NADPH.

Higher-plant PSI is a multi-protein pigment complex, composed of two moieties: the core and the LHCI (peripheral light-harvesting antenna). The core complex harbours the RC (reaction centre), all of the electron transport cofactors, ~100 Chl *a* (chlorophyll *a*) and 22  $\beta$ -carotenes [1,2]. LHCI co-ordinates Chl *a*, Chl *b*, and the Cars (carotenoids)  $\beta$ -carotene, violaxanthin and lutein [3]. It is composed of four Lhcs (light-harvesting complexes), called Lhca1–Lhca4. The Lhca proteins are encoded by the Lhc gene family, which also includes the Lhcb antenna complexes of PSII. All Lhcs show high sequence conservation, with 43–55 % sequence identity between the different Lhcas and up to 75 % sequence homology [4]. The Lhcas are located on one side of the core and are assembled into two dimers: Lhca1/4 and Lhca2/3 [5,6]. It has been suggested that under certain growth conditions Lhca2 and Lhca3 might be able to form homodimers [1]. However, it has been shown that all four Lhca proteins are present in a 1:1 ratio with the PSI core under standard- [7], high- and low-light conditions [8], in agreement with the exclusive presence of a heterodimeric Lhca2/3 complex.

The main function of LHCI is to harvest light and transfer the excitation energy to the RC, where it is used for charge separation. In addition, it has been shown that the LHCI moiety is the first target of high-light damage of PSI–LHCI, thereby protecting the core complex against photodamage [9–11].

A peculiar feature of PSI is the presence of red forms: Chls that absorb at energy lower than that of the primary electron

donor, P700, and that have extremely broad and red-shifted fluorescence emission spectra [12]. They are conserved in plants, algae and bacteria. Their function is still not fully understood. It has been suggested that they: (i) focus the energy to the primary electron donor, (ii) have a role in protection against light-stress, or (iii) absorb light efficiently in a dense vegetation system where light is enriched in wavelengths above 690 nm [13,14]. It has been shown that the red forms have an important effect on the excitation-energy transfer of PSI [12,15,16]. Being at low energy, these Chls have a high probability of being populated [17] and their excitation energy must be transferred energetically uphill to P700, in order to be used for photochemistry [16]. In higher plants, the red forms are associated with LHCI. Although several studies have analysed the trapping kinetics in PSI, no general agreement has been reached [15,18,19]. This is mainly because PSI is a very large and complex system and little information is at present available about the properties of the individual PSI building blocks (e.g. Lhca dimers). This information is necessary to be able to disentangle the contribution of the individual complexes from the analysis of the whole system.

In the past, LHCI was often separated into two fractions upon isolation: LHCI-680 and LHCI-730, named after their LT [low temperature (77 K)] fluorescence emission maxima [20–24]. LHCI-680 lacks the red-shifted emission and consists mainly of monomeric Lhca2 and Lhca3, whereas LHCI-730 is strongly enriched in the Lhca1/4 heterodimer [21,23,24]. On the basis of these results, it was assumed that only the Lhca1/4 dimer contains ‘red’ Chls. However, the LT 680 nm fluorescence was absent from preparations containing all four Lhca complexes in their native dimeric form [25,26]. Furthermore, dimeric fractions enriched in Lhca1/4 or Lhca2/3 both showed a red-absorption tail with similar amplitude, indicating that red forms are also present in Lhca2/3 [27].

Abbreviations used:  $\beta$ -DM,  $\beta$ -D-maltoside; Car, carotenoid; Chl, chlorophyll; FWHM, full width at half maximum; Lhc, light-harvesting complex; LHCI, peripheral light-harvesting antenna; LT, low temperature; PS, Photosystem; RC, reaction centre; RT, room temperature; T-DNA, transferred DNA; WT, wild-type.

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Owing to the very similar biochemical properties of the two dimeric LHCI complexes, full separation has not been achieved yet and most of the available information on the individual Lhca complexes was obtained by *in vitro* reconstitution of monomeric Lhca1–Lhca4 and dimeric Lhca1/4 antenna complexes [23, 27–29]. The LT fluorescence-emission maxima were found at 690 nm (Lhca1), 702 nm (Lhca2), 725 nm (Lhca3) and 733 nm (Lhca4) [27–29]. It has been shown that the red forms in the Lhca complexes originate from a strongly excitonically coupled Chl dimer, involving Chl603 and Chl609 (nomenclature as in [30]). It has also been demonstrated that an asparagine residue as a ligand for Chl603, as it is in Lhca3 and Lhca4, is needed for this strong coupling. Indeed, all other Lhc complexes have a histidine residue at this position and do not show red-shifted emission [31–35]. To account for the extreme red-shift and the broad fluorescence-emission spectra, it was suggested that the lowest exciton state of the dimer mixes with a charge-transfer state [36–38], which was recently demonstrated to be correct in the case of the Lhca4 monomer [39].

In the present study we purified and fully characterized the Lhca2/3 and Lhca1/4 heterodimers, obtaining a complete picture of their biochemical, spectroscopic and functional properties, thus opening the way to a full understanding of the excitation-energy transfer in PSI. We also show that LHCI-680 is not a native state of the antenna complexes of PSI. Finally, the availability of native purified complexes allows us to compare them with the recombinant antenna complexes.

## EXPERIMENTAL

### Plant material

The WT (wild-type), Lhca1 T-DNA (transferred DNA) knockout ( $\Delta a1$ ) and Lhca2 antisense ( $\Delta a2$ ) *Arabidopsis thaliana* (WT-col-0) plants (described in [40,41]) were grown at a 8 h light/16 h dark regime of 22°C/19°C, with a light intensity of 130  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  and 70 % relative humidity.

### LHCI isolation and analysis

Thylakoids were isolated as described previously [20]. PSI–LHCI isolation was as described in [42]. LHCI isolation was adapted from [25]. In short, PSI–LHCI with a Chl concentration of 0.3 mg/ml was solubilized with 1 % n-dodecyl  $\beta$ -DM ( $\beta$ -D-maltoside) and 0.5 % Zwittergent-16. After 1 min of vortex-mixing, the sample was loaded on to a 0.1–1 M sucrose gradient, containing 10 mM Tricine, pH 7.8, and 0.03 %  $\beta$ -DM and centrifuged at 41 000 rev./min for 22 h in a Beckman SW41 rotor. The fractions were analysed by a modified Laemmli SDS/PAGE (15.5 % gel) system and Coomassie Blue staining, as described in [7]. Quantities loaded on a Chl basis were: 3  $\mu\text{g}$  of PSI–LHCI, 1  $\mu\text{g}$  of dimeric fraction of solubilized PSI–WT and 0.5  $\mu\text{g}$  of the dimeric fraction of PSI– $\Delta a1$  and  $\Delta a2$ . Quantification of Coomassie Blue staining was carried out as described in [7] after digitizing the gel with a Fujifilm LAS 3000 scanner. Immunoblotting was carried out as described in [42]. Pigment analysis was carried out as in [43].

### Steady-state spectroscopy

Absorption spectra were recorded on a Varian Cary 4000 UV–visible spectrophotometer. For 77 K measurements, a home-built liquid-nitrogen cooled low-temperature device was used. Fluorescence spectra were recorded at 77 K and 283 K on a

Fluorolog 3.22 spectrofluorimeter (Jobin Yvon-Spex). Samples were diluted to an absorbance of 0.04  $\text{cm}^{-1}$  at 680 nm. CD spectra were recorded at 283 K on an AVIV 62ADS spectropolarimeter. All measurements were performed in 10 mM Tricine, pH 7.8, 0.03 %  $\alpha$ -DM and 0.5 M sucrose [RT (room temperature; 293 K) and 283 K] or 67 % (w/v) glycerol for 77 K measurements.

### Fluorescence quantum yield

Fluorescence quantum yields ( $\Phi_F$ ) at 283 K were calculated by dividing the ratio of integrated fluorescence intensities, on a wavenumber scale, by the ratio of their total absorption factor ( $1 - \text{Transmission}$ ) for the spectral region around 630 nm, where the sample was excited. The emission of Chl *a* in acetone, with  $\Phi_F = 0.30$  [44], was used as a reference.

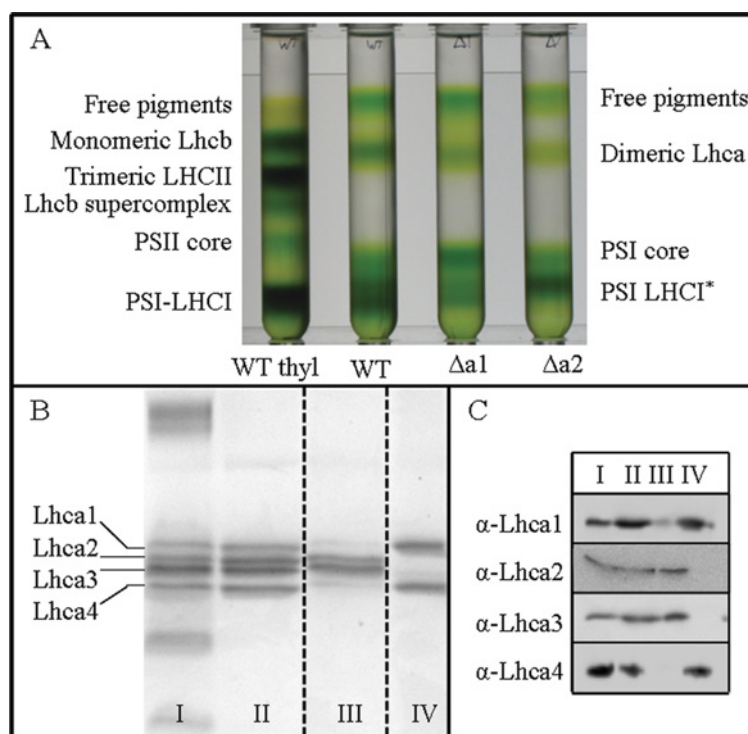
### Photobleach assay

Lhca dimers were illuminated for 20 min in the presence of oxygen at RT with a Schott 200 cold-white-light source at stage 3, giving a light intensity of 5.5  $\text{mE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Light was passed through a 1 cm water filter. Samples in a 4 mm  $\times$  10 mm cuvette, with an initial absorbance at  $Q_y$  maximum of 0.3 in the light path of 4 mm, were homogenized every 2 min. Control samples were kept at RT in the dark for 20 min. Samples were concentrated, loaded on a 0.1–1 M sucrose gradient and centrifuged at 44 000 rev./min for 19 h in a Beckman SW60 rotor.

## RESULTS

### Isolation of the native LHCI dimers

LHCI of WT plants is suggested to be composed of two dimers: Lhca2/3 and Lhca1/4 [6]. However, homogeneous purification of these dimers has never been achieved from PSI–WT because of the highly similar properties of the complexes [27]. It has been shown recently [42] that PSI complexes, co-ordinating only Lhca2/3 or Lhca1/4, can be obtained from plants lacking either Lhca1 or Lhca2 ( $\Delta a1$  and  $\Delta a2$  *Arabidopsis* mutants). These preparations thus represent a good starting point for the homogeneous preparation of each dimer. PSI– $\Delta a1$ ,  $\Delta a2$  and WT were solubilized and subjected to sucrose density gradient ultracentrifugation (Figure 1A). Five pigment-containing fractions were obtained and identified by their mobility in the gradient, by SDS/PAGE and by absorption spectroscopy (results not shown) as free pigments, dimeric Lhca, PSI core and PSI supercomplexes with reduced (PSI–LHCI\*) or with full (PSI–LHCI) antenna size. In the gradient of PSI– $\Delta a1$ , which, in addition to Lhca2 and Lhca3, also retained part of Lhca4 [42], some monomeric Lhca4 was present between the free pigments and dimeric Lhca. The SDS/PAGE and immunoblot analysis (Figures 1B and 1C) of the dimeric Lhca fraction purified from the PSI– $\Delta a1$ , PSI– $\Delta a2$  and PSI–WT preparations shows that they contain respectively Lhca2/3, Lhca1/4 and a mixture of the two dimers (in the following named LHCI). The SDS/PAGE gel shows that the Lhca1/4 dimer is 100 % pure, whereas the purity of the Lhca2/3 dimer is 95 % (Supplementary Table S1 at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>), the impurity being Lhca1/4, as expected, because a small amount of Lhca1/4 dimer is present in the starting PSI preparation, as shown previously [42]. This is due to the fact that the T-DNA insertion of the  $\Delta a1$  plants is located in the promoter region, and thus a small part of the PSI complexes in these plants retains Lhca1 [45] and thus the Lhca1/4 dimer. The analysis of the gel (Supplementary Figure S1



**Figure 1** PSI solubilization

(A) Sucrose gradients of solubilized PSI from WT,  $\Delta a1$  and  $\Delta a2$  plants. In addition, a sucrose gradient of mildly solubilized WT thylakoids (thyl) is presented to show the migration behaviour of Lhc complexes in monomeric and trimeric aggregation state. SDS/PAGE (B) and immunoblot (C) analysis of PSI-LHCI (I) and dimeric Lhca fraction of solubilized PSI from WT (II),  $\Delta a1$  (III) and  $\Delta a2$  (IV) plants. Note that in the case of PSI-LHCI, Psd and Lhca3 overlap on this gel.

**Table 1** Pigment-binding properties of Lhca dimers

The pigment compositions of LHCI, Lhca1/4, Lhca2/3 and the average of Lhca1/4 and Lhca2/3 are normalized to 100 Chls. Values are the means of three measurements on independent preparations for Lhca1/4 and Lhca2/3 and of two repetitions for LHCI. S.D. is indicated in parentheses.

	Chl <i>a/b</i>	Chl/Car	Violaxanthin	Lutein	$\beta$ -Car
LHCI	3.71 (0.01)	4.65 (0.01)	4.74 (0.04)	11.14 (0.02)	5.39 (0.03)
Lhca1/4	3.74 (0.09)	4.82 (0.04)	5.30 (0.05)	11.31 (0.13)	4.23 (0.13)
Lhca2/3	3.70 (0.12)	4.69 (0.07)	4.07 (0.15)	10.55 (0.35)	6.66 (0.46)
Mean	3.72	4.77	4.69	10.9	5.45

at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>) also showed that Lhca2 and Lhca3 are present in equal amounts, confirming that they are present as a heterodimer.

### Pigment composition

The pigment composition of the Lhca1/4 and Lhca2/3 dimers is very similar (Table 1), both having a Chl *a/b* ratio of 1:3.7 and a Chl/Car ratio of 1:4.7–4.8. The main difference concerns the ratio between the Cars: the amount of  $\beta$ -carotene is higher in Lhca2/3 than in Lhca1/4, whereas the opposite is true for violaxanthin and lutein.

### LT absorption

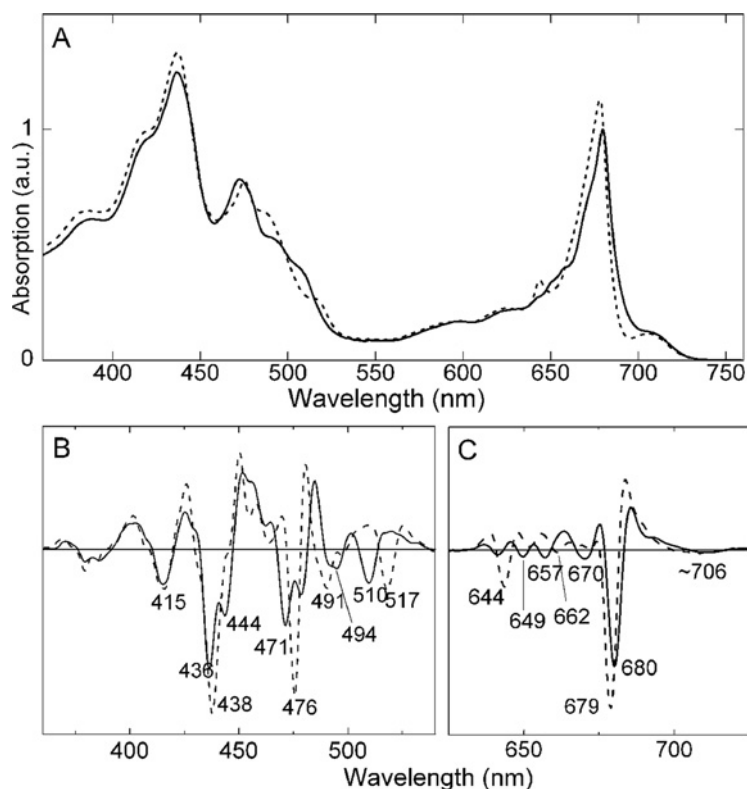
The 77 K absorption spectra (Figure 2A) of the two dimers show clear differences reflecting the different environment in which

the pigments are embedded as can be appreciated from the second-derivative analysis of the spectra (Figures 2B and 2C). It is important to underline that, in several regions in which the second derivative of Lhca1/4 shows a signal, no signal is present for Lhca2/3, indicating that the 5% impurity (Lhca1/4) in the latter preparation is below the detection sensitivity and does not influence our measurements.

The most peculiar feature of Lhca complexes is the presence of low-energy-absorption forms. Interestingly, the absorption in the red tail is extremely similar for both dimers. In order to obtain more details about the red forms, the  $Q_y$  region of the spectra was described in terms of Gaussians (Supplementary Figure S2 at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>). The red-most bands show a maximum at 706–707 nm and a FWHM (full width at half maximum) of 25 nm and they represent 8.5% and 8.9% of the total oscillator strength in the  $Q_y$  region (630–750 nm) for Lhca1/4 and Lhca2/3 respectively. The high similarity of the absorption properties indicates that the organization of the Chls responsible for the red absorption is also very similar in the two dimers.

### CD

In order to compare the pigment–pigment interactions in Lhca1/4 and Lhca2/3, the CD spectra were recorded (Figure 3A). In the  $Q_y$  region, the main components of both dimers have the characteristic (– + –) signal like all other members of the Lhc family [46], indicating a similar structural arrangement of the pigments. In the blue region, the spectra are rather different, indicating specific pigment–pigment or pigment–protein interactions in the two dimers.



**Figure 2** LT absorption of Lhca1/4 and Lhca2/3

(A) Absorption spectra (77 K) of Lhca1/4 (broken line) and Lhca2/3 (continuous line). Spectra are normalized to Chl content. Second derivatives of the absorption spectra in the Soret (B) and  $Q_y$  (C) region.

### Steady-state fluorescence

The fluorescence-emission spectra of the two dimers, recorded at 283 K and 77 K, are presented in Figures 3(B) and 3(C). At 283 K, the Lhca1/4 spectrum has maxima at 685.5 and 721 nm. Lhca2/3 has a similar spectral shape with maxima at 687 and 713 nm. Upon lowering the temperature to 77 K, the Lhca1/4 dimer loses nearly all 685.5 nm emission, whereas the maximum shifts to 731.5 nm. The maximum of Lhca2/3 also shifts to lower energies (728.5 nm), however, a shoulder remains around 697 nm. An emission band around 700 nm was observed previously for an LHCI preparation containing both Lhca dimers [25,26], and was assigned to Lhca2 [28]. This is puzzling, because for an equilibrated intact Lhca2/3 dimer at 77 K, nearly all excitation energy is expected to be located on the lowest-energy form of Lhca3 [28]. A possible explanation for the observed emission could be that some dissociation of dimers had occurred, giving rise to Lhca2 that could not transfer its energy to Lhca3. To investigate whether this was the case, the oligomeric state of the sample was analysed on a sucrose gradient. Only one band was observed (result not shown), and its LT fluorescence spectrum was recorded directly after harvesting. The 697 nm emission was still present, indicating that it is an intrinsic property of the dimeric complex.

Previous data on recombinant complexes have shown that the fluorescence yield of Lhca complexes is far lower than that of LHCII, thus indicating that Lhca complexes are in a partially quenched state in solution [37]. This is particularly interesting because a quenched conformation can be related to the mechanisms of energy dissipation in the antenna (for a review see [47]), which protect the plants against high-light damage. To check whether this is also the case for native dimeric Lhca, the

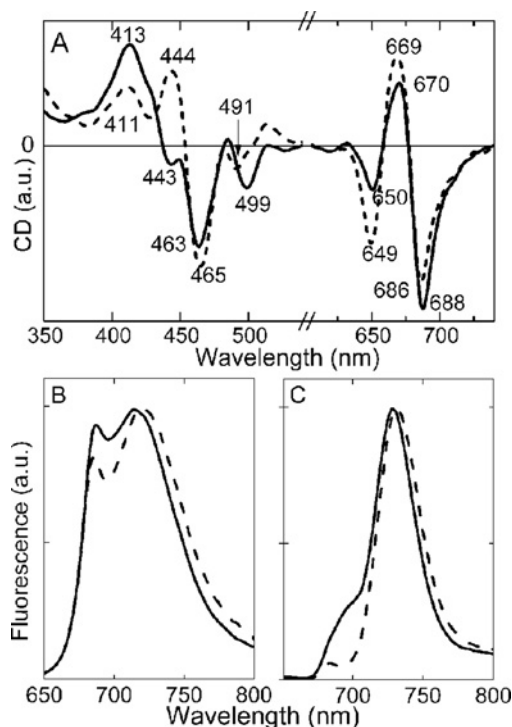
fluorescence yield ( $\Phi_F$ ) at 283 K was calculated.  $\Phi_F$  was 0.15 ( $\pm 0.01$ ) for Lhca2/3 and 0.14 ( $\pm 0.01$ ) for Lhca1/4. These values for the native dimers are significantly higher than the value of 0.063 reported for the reconstituted Lhca1/4 complex [37], but lower than that of LHCII ( $\Phi_F = 0.22$  [48]), indicating that the Lhca dimers are indeed in a partially quenched state in solution.

### Comparing the properties of Lhca1/4 and Lhca2/3 with those of LHCI

The peripheral antenna complex of PSI (LHCI) is composed of equal amounts of Lhca1/4 and Lhca2/3 [7]. Therefore the normalized spectra of the purified Lhca1/4 and Lhca2/3 dimer should add up to the spectra of LHCI. Figure 4 shows the comparison of the sum of the absorption, CD and fluorescence spectra of the two dimers with the spectra of the LHCI fraction purified from WT plants. The excellent agreement shows that indeed the spectroscopic properties of LHCI can be explained by those of the two purified dimers.

### Comparing the dimers with the intact system

In order to investigate whether the properties of the dimers change during purification, their LT emission is compared with those in isolated PSI systems and PSI in the thylakoid membranes. In PSI- $\Delta a3$ , Lhca3 is completely lacking, therefore Lhca4 coordinates the lowest-energy Chls and is responsible for most of the LT emission. This allows comparison of the spectroscopic properties of the red forms of Lhca4 in PSI with those of Lhca1/4



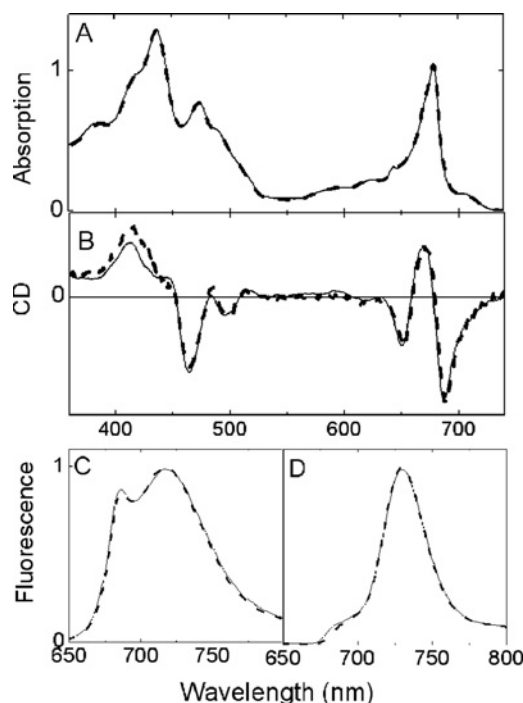
**Figure 3** CD and fluorescence of the dimeric Lhca complexes

(A) CD spectra of Lhca1/4 (broken line) and Lhca2/3 (continuous line) at 283 K. Spectra are normalized to the Chl concentration. Fluorescence emission spectra of Lhca1/4 (broken line) and Lhca2/3 (continuous line) at (B) 283 K and (C) 77 K. Excitation was at 475 nm; spectra are normalized to the maxima.

(Figure 5A). The properties of the red forms in Lhca3 (of Lhca2/3) can be compared with those of  $\Delta a4$ -PSI, where Lhca4 is replaced by Lhca5 [42]. In this complex, Lhca3 is responsible for most of the LT emission (Figure 5B). In both cases, there is a good match between the spectra compared. This means that the properties of the red forms remained unchanged during the purification of the Lhca1/4 and Lhca2/3 dimer. This is confirmed further by the comparison of the spectra of the dimers with that of the thylakoid membrane (Figure 5C). Also in this case there is a good overlap between the spectra of the dimers and the reddest peak of the thylakoid spectrum which originates from PSI. Because the red forms are very sensitive to changes in the environment of the corresponding pigments [49], the fact that LT emission does not change upon isolation indicates that the dimers are in their native state.

### Photobleaching

It has been shown that the LHCI moiety is the first target of high-light damage in PSI-LHCI [9–11]. This was explained by the concentration of excitation energy on the red forms, thus giving rise to the highest probability of generating dangerous triplets on the corresponding Chls [9], which are mainly located in LHCI [25]. However, it has also been reported that triplets formed on the red forms are quenched with 100% efficiency by a nearby Car located in the 621 site [50], which would provide excellent photoprotection to the whole system. Thus there is a discrepancy: on the one hand, the red forms are proposed as a site of photodamage, but on the other, they were shown to be fully protected. To clarify this point, we investigated the photodamage for isolated dimers.



**Figure 4** Comparison of the spectra of Lhca1/4 and Lhca2/3 (continuous line) with those of LHCI (broken line)

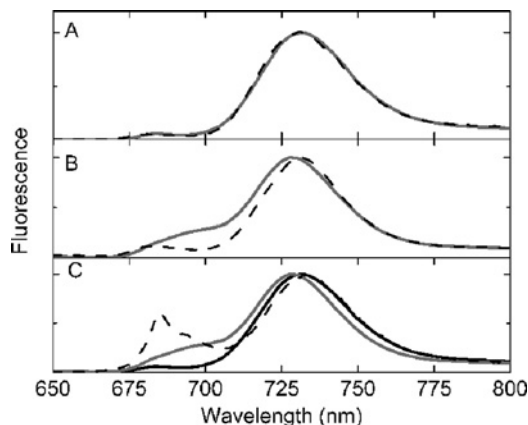
(A) Sum of LT absorption spectra of Lhca1/4 and Lhca2/3, compared with LHCI. Spectra are normalized in the  $Q_y$  region to the number of Chls. (B) Sum of CD spectra of Lhca1/4 and Lhca2/3 compared with that of LHCI. (C) 283 K fluorescence emission spectra of summed Lhca1/4, summed Lhca2/3 and LHCI. Spectra are normalized on an energy scale to their fluorescence quantum yield. For LHCI, a value of 14.5% was used. (D) LT fluorescence emission spectra of summed Lhca1/4, summed Lhca2/3 and LHCI. Spectra are normalized to the same fluorescence quantum yield.

Both dimers were subjected to high-light treatment. The absorption spectra of the treated and untreated samples are presented in Figure 6. The total absorption in the  $Q_y$  region decreased, demonstrating that photobleaching had occurred. The difference spectra (untreated–treated) of both dimers show a peak at 682 nm, a shoulder at 672 nm and a tail extending into the red, meaning that the red forms are not fully protected.

A light-induced trimer–monomer transition has been observed for the major light-harvesting complex of PSII (LHCII) [51]. Therefore it was investigated whether light-induced monomerization also occurs in the Lhca dimers. The complexes were exposed to strong light followed by density-gradient centrifugation. Interestingly, light-induced monomerization was observed for Lhca2/3, but not for Lhca1/4 (Figure 7).

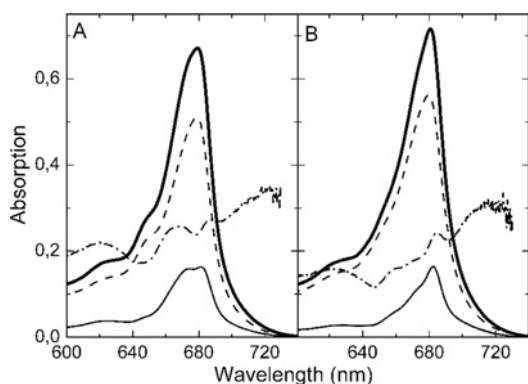
### Monomerization affects pigment organization

For recombinant Lhca1/4, it has been shown that dimerization affects the pigment interactions, as indicated by the fact that the CD spectrum of the dimer differs from the sum of the CD spectra of the monomers [27]. The light-induced monomerization of the Lhca2/3 dimer (Figure 7) allowed us to investigate whether a similar effect occurs in these complexes. Figure 8 shows that the CD spectra of the monomeric band, containing Lhca2 and Lhca3, is rather different from the dimer. Therefore it can be concluded that dimerization affects the pigment organization in both dimers and/or creates new interactions between pigments of different monomers.



**Figure 5** Fluorescence emission of Lhca dimers compared with PSI

LT emission spectra of Lhca1/4 (grey continuous line) and  $\Delta a3$ -PSI (black broken line) (A), Lhca2/3 (grey continuous line) and  $\Delta a4$ -PSI (black broken line) (B) and Lhca1/4 (black continuous line), Lhca2/3 (grey continuous line) and thylakoids (black broken line) (C). Thylakoids were of Lhcb2 antisense plants which have a reduced LHCII antenna size and therefore the PSI emission is less obscured by PSII emission. Spectra are normalized to the maxima.

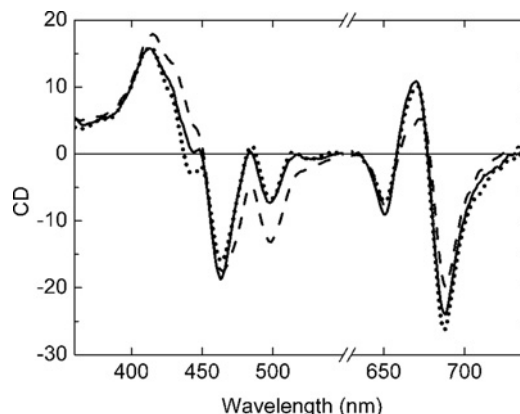


**Figure 6** Photobleaching of native Lhca dimers

Absorption spectra of Lhca1/4 (A) and Lhca2/3 (B); control (thick continuous line), light-treated sample (dotted line), difference (thin continuous line), and the ratio difference/control (dashed-dotted line).

#### Reconstituted compared with native Lhca complexes

The availability of native dimers and monomers of Lhca allows for a comparison of their properties with those of the reconstituted complexes (Supplementary Figure S3 at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>).



**Figure 8** CD spectra of monomeric compared with dimeric Lhca2/3

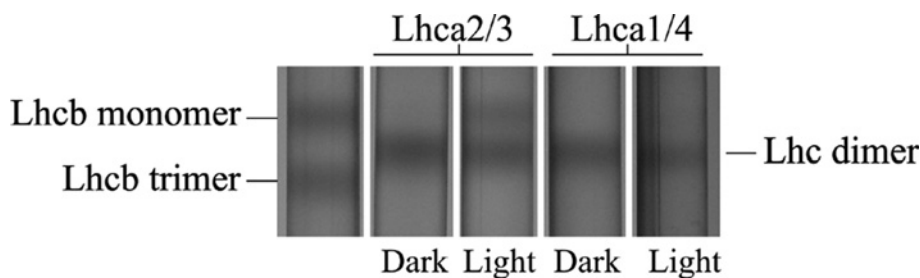
CD spectra of dimeric Lhca2/3 before (dotted line) and after (continuous line) light treatment, and a light induced mixture of monomeric Lhca2 and Lhca3 (broken line). Spectra are normalized to the absorption in the Q<sub>y</sub> region.

BiochemJ.org/bj/433/bj4330477add.htm), which have been widely used for the study of the PSI antenna complexes.

On the basis of the pigment composition of the monomers, identical Chl *a/b* ratio and Chl/Car ratios are expected in both dimers, as is indeed the case (Supplementary Table S2 at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>). The Car composition of the sum of the monomers is similar to that of the dimer, indicating that the specificity for the Car binding is maintained in the reconstituted complexes. The only difference is the higher amount of  $\beta$ -carotene (at the cost of lutein) in the dimers. This was also observed for the reconstituted Lhca1/4 dimer, indicating that dimerization stabilizes the co-ordination of  $\beta$ -carotene, or that the binding of  $\beta$ -carotene is required for dimerization, but that this difference is not due to the reconstitution procedure.

The data also show high similarity between the spectroscopic properties of the native and reconstituted Lhca1/4 dimers (Supplementary Figure S3 at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>). The properties of the red-most bands are identical, although the reconstituted complex shows somewhat smaller intensity in the red band.

So far it has not been possible to reconstitute the Lhca2/3 dimer [23], but the individual monomers were obtained [23,28]. In Supplementary Figure S4 (at <http://www.BiochemJ.org/bj/433/bj4330477add.htm>) the sum of the reconstituted Lhca2 and Lhca3 spectra is compared with the mixture of native Lhca2 and Lhca3 obtained by light-induced monomerization. The



**Figure 7** Analysis of oligomeric state of light-treated Lhca complexes

Sucrose density gradients loaded with Lhca2/3 and Lhca1/4 and kept for 20 min at RT in the dark or in strong light. The left gradient was loaded with a mixture of monomeric and trimeric Lhcb complexes.



striking similarity of the spectra shows that also in this case the pigment organization in the reconstituted complexes is virtually identical to that of the native ones.

## DISCUSSION

### LHCI-680 is not a natural component of the PSI antenna complex

For a long time, it has been thought that LHCI is composed of two fractions: one without red forms, LHCI-680 (enriched in Lhca2 and Lhca3), and one with red forms, LHCI-730 (enriched in Lhca1 and Lhca4). However, it was also shown that fractions containing all four Lhca complexes in a dimeric state did not show emission at 680 nm at LT and it was inferred that the LHCI-680 fraction was composed of partially denatured complexes in a monomeric state [27,52]. Therefore it was suggested that native LHCI is composed of dimeric complexes which all contain red forms [27]. Unfortunately, these complexes could never be fully separated. In the present study, by using PSI complexes from mutant plants, we were able for the first time to purify the Lhca1/4 and Lhca2/3 dimeric complexes. The properties of these dimers can fully explain the pigment composition (Table 1) and CD, absorption and fluorescence spectra (Figure 4) of LHCI. Moreover, both dimers show red emission forms with maxima around 730 nm, the same as in the PSI complex, implying that the purification does not alter the properties of the dimers. These results are the final proof that LHCI-680 is not a natural component of the PSI antenna complex.

### Lhca2 and Lhca3 form a functional heterodimer

It is well established that Lhca1 and Lhca4 form a heterodimer, whereas the heterodimeric association state of Lhca2 and Lhca3 could not be confirmed by either reconstitution *in vitro* [23] or purification of the complex [27]. Several results indicate that Lhca2 and Lhca3 are not associated with the PSI core as a homodimer: (i) In Lhca2 antisense plants Lhca3 is completely lacking at the PSI level, whereas its mRNA is present [41,42,45,53], thus demonstrating that an Lhca3 homodimer is not being formed. (ii) In the Lhca3 antisense plants 35 % of Lhca2 (as compared with the WT level) is still associated with the core, but exclusively in its monomeric form as is evident from both electron microscopy and sucrose-density-gradient ultracentrifugation, where Lhca2 was only found in the monomeric fraction [42]. In the present study, we were able to isolate Lhca2/3 and to show that it is a functional heterodimer in which energy transfer between the monomers occurs, as indicated by the fact that most of the emission is red-shifted, thus originating from Lhca3 [27–29].

### Four different Lhca complexes form two nearly identical dimers

The four monomeric Lhcas obtained by *in vitro* reconstitution differ strongly from each other in their biochemical and spectroscopic properties [37,54]. However, it was noticed previously [53] that the sum of the absorption spectra of reconstituted monomeric Lhca1 and Lhca4 was similar to the sum of Lhca2 and Lhca3, therefore it was expected that the two dimers would also have similar absorption properties. The analysis of the native complexes shows that this is indeed the case: the two dimers have identical Chl *a/b* ratios and rather similar absorption spectra. What is surprising is that this similarity is not limited to the absorption, but it extends to most of the spectroscopic properties including long-wavelength emission and fluorescence quantum yield ( $\Phi_F$ ). From a functional point of view, PSI thus has

two identical antenna units thereby extending the pseudo-binary symmetry, which characterizes the core complex [2], to the outer antenna system.

Although light-harvesting represents the main task of Lhca complexes, they are also involved in photoprotection: under high-light conditions they can act as a 'fuse' and dissipate excess energy to minimize photodamage to the core complex [9]. In this respect, the data show a clear difference in functionality for the two dimers: light treatment leads to the monomerization of Lhca2/3 but not of Lhca1/4. In PSI-LHCI, monomerization of Lhca2/3 might induce the dissociation of the dimer from the core complex. In this case, Lhca2/3 cannot transfer its excitation energy to P700, which leads to an increase of the excited-state lifetime and thus to an increased probability to form Chl triplets. This can thus explain why Lhca2 and Lhca3 are the first antennas to be degraded upon light treatment of PSI-LHCI [11,55,56].

### The domain harbouring the red forms is conserved in the two dimers

A peculiar feature of the antenna complexes of PSI is the presence of Chls that absorb above 700 nm and which are associated with Lhca3 and Lhca4 [36]. The properties of these forms could be studied for native and functional dimers, allowing a direct comparison of Lhca2/3 with Lhca1/4 (Supplementary Figure S4). The data show that the red forms have nearly identical spectroscopic properties in both dimers, indicating that their environment and organization must be very similar.

### The two dimers have identical light-harvesting properties: implications for energy transfer in PSI-LHCI

The study of excitation-energy transfer and trapping in PSI is extremely complex because the system is composed of 170 Chl molecules, making the modelling of the fluorescence kinetics very challenging. The system is usually described using compartment models in which the major building blocks (Lhca complexes, core complex and the RC) are considered. Knowledge about the spectroscopic properties of the compartments is required for the evaluation of these models [18]. Until now this information was not available. The newly obtained data for the dimers open the way to design and evaluate a model which truly describes the energy trapping and transfer in PSI.

### The reconstituted complexes are good replicas of the native system

In the present study, the biochemical and spectroscopic properties of native and reconstituted complexes were compared. The data clearly demonstrate that all of the major properties of the monomeric Lhcas are reproduced in the *in vitro* systems, testifying once more that the reconstituted complexes are valuable replicas of the native systems.

## AUTHOR CONTRIBUTION

Emilie Wientjes and Roberta Croce designed the experiments and wrote the paper. Emilie Wientjes performed all experiments.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Amunts, A., Drory, O. and Nelson, N. (2007) The structure of a plant Photosystem I supercomplex at 3.4 Å resolution. *Nature* **447**, 58–63
- Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. and Krauss, N. (2001) Three-dimensional structure of cyanobacterial Photosystem I at 2.5 Å resolution. *Nature* **411**, 909–917
- Croce, R. and Bassi, R. (1998) The light-harvesting complex of photosystem I: pigment composition and stoichiometry. In *Photosynthesis: Mechanisms and Effects* (Garab, G., ed.), pp 421–424. Kluwer Academic Publishers, Dordrecht
- Jansson, S. (1999) A guide to the *Lhc* genes and their relatives in *Arabidopsis*. *Trends Plant Sci.* **4**, 236–240
- Boekema, E.J., Jensen, P.E., Schlodder, E., van Breemen, J.F.L., van Roon, H., Scheller, H.V. and Dekker, J.P. (2001) Green plant Photosystem I binds light-harvesting complex I on one side of the complex. *Biochemistry* **40**, 1029–1036
- Ben-Shem, A., Frolow, F. and Nelson, N. (2003) Crystal structure of plant Photosystem I. *Nature* **426**, 630–635
- Ballottari, M., Govoni, C., Caffarri, S. and Morosinotto, T. (2004) Stoichiometry of LHCl antenna polypeptides and characterization of gap and linker pigments in higher plants Photosystem I. *Eur. J. Biochem.* **271**, 4659–4665
- Ballottari, M., Dall'Osto, L., Morosinotto, T. and Bassi, R. (2007) Contrasting behavior of higher plant Photosystem I and II antenna systems during acclimation. *J. Biol. Chem.* **282**, 8947–8958
- Alboresi, A., Ballottari, M., Hiennerwadel, R., Giacometti, G.M. and Morosinotto, T. (2009) Antenna complexes protect Photosystem I from photoinhibition. *BMC Plant Biol.* **9**, 71
- Andreeva, A., Abarova, S., Stoitchkova, K., Picorel, R. and Velitchkova, M. (2007) Selective photobleaching of chlorophylls and carotenoids in Photosystem I particles under high-light treatment. *Photochem. Photobiol.* **83**, 1301–1307
- Hui, Y., Jie, W. and Carpentier, R. (2000) Degradation of the Photosystem I complex during photoinhibition. *Photochem. Photobiol.* **72**, 508–512
- Gobets, B. and van Grondelle, R. (2001) Energy transfer and trapping in Photosystem I. *Biochim. Biophys. Acta* **1507**, 80–99
- Rivadossi, A., Zucchelli, G., Garlaschi, F.M. and Jennings, R.C. (1999) The importance of PSI chlorophyll red forms in light-harvesting by leaves. *Photosyn. Res.* **60**, 209–215
- Trissl, H.W. (1993) Long-wavelength absorbing antenna pigments and heterogeneous absorption-bands concentrate excitons and increase absorption cross-section. *Photosyn. Res.* **35**, 247–263
- Ihalainen, J.A., van Stokkum, I.H.M., Gibasiewicz, K., Germano, M., van Grondelle, R. and Dekker, J.P. (2005) Kinetics of excitation trapping in intact Photosystem I of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*. *Biochim. Biophys. Acta* **1706**, 267–275
- Jennings, R.C., Zucchelli, G., Croce, R. and Garlaschi, F.M. (2003) The photochemical trapping rate from red spectral states in PSI-LHCl is determined by thermal activation of energy transfer to bulk chlorophylls. *Biochim. Biophys. Acta* **1557**, 91–98
- Croce, R., Zucchelli, G., Garlaschi, F.M., Bassi, R. and Jennings, R.C. (1996) Excited state equilibration in the Photosystem I light-harvesting I complex: P700 is almost isoenergetic with its antenna. *Biochemistry* **35**, 8572–8579
- Slavov, C., Ballottari, M., Morosinotto, T., Bassi, R. and Holzwarth, A.R. (2008) Trap-limited charge separation kinetics in higher plant Photosystem I complexes. *Biophys. J.* **94**, 3601–3612
- van Oort, B., Amunts, A., Borst, J.W., van Hoek, A., Nelson, N., van Amerongen, H. and Croce, R. (2008) Picosecond fluorescence of intact and dissolved PSI-LHCl crystals. *Biophys. J.* **95**, 5851–5861
- Bassi, R. and Simpson, D. (1987) Chlorophyll–protein complexes of barley Photosystem-I. *Eur. J. Biochem.* **163**, 221–230
- Knoetzel, J., Svendsen, I. and Simpson, D.J. (1992) Identification of the Photosystem I antenna polypeptides in barley: isolation of three pigment-binding antenna complexes. *Eur. J. Biochem.* **206**, 209–215
- Lam, E., Ortiz, W. and Malkin, R. (1984) Chlorophyll a/b proteins of Photosystem I. *FEBS Lett.* **168**, 10–14
- Schmid, V.H.R., Potthast, S., Wiener, M., Bergauer, V., Paulsen, H. and Storf, S. (2002) Pigment binding of Photosystem I light-harvesting proteins. *J. Biol. Chem.* **277**, 37307–37314
- Tjss, S.E., Roobolboza, M., Palsson, L.O. and Andersson, B. (1995) Rapid isolation of Photosystem I chlorophyll-binding proteins by anion-exchange perfusion chromatography. *Photosyn. Res.* **45**, 41–49
- Croce, R., Zucchelli, G., Garlaschi, F.M. and Jennings, R.C. (1998) A thermal broadening study of the antenna chlorophylls in PSI-200, LHCl, and PSI core. *Biochemistry* **37**, 17355–17360
- Ihalainen, J.A., Gobets, B., Sznee, K., Brazzoli, M., Croce, R., Bassi, R., van Grondelle, R., Korppi-Tommola, J.E.I. and Dekker, J.P. (2000) Evidence for two spectroscopically different dimers of light-harvesting complex I from green plants. *Biochemistry* **39**, 8625–8631
- Croce, R., Morosinotto, T., Castelletti, S., Breton, J. and Bassi, R. (2002) The Lhca antenna complexes of higher plants photosystem I. *Biochim. Biophys. Acta* **1556**, 29–40
- Castelletti, S., Morosinotto, T., Robert, B., Caffarri, S., Bassi, R. and Croce, R. (2003) Recombinant Lhca2 and Lhca3 subunits of the Photosystem I antenna system. *Biochemistry* **42**, 4226–4234
- Schmid, V.H.R., Cammarata, K.V., Bruns, B.U. and Schmidt, G.W. (1997) *In vitro* reconstitution of the Photosystem I light-harvesting complex LHCl-730: heterodimerization is required for antenna pigment organization. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7667–7672
- Liu, Z., Yan, H., Wang, K., Kuang, T., Zhang, J., Gui, L., An, X. and Chang, W. (2004) Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution. *Nature* **428**, 287–292
- Croce, R., Morosinotto, T., Ihalainen, J.A., Chojnicka, A., Breton, J., Dekker, J.P., van Grondelle, R. and Bassi, R. (2004) Origin of the 701 nm fluorescence emission of the Lhca2 subunit of higher plant Photosystem I. *J. Biol. Chem.* **279**, 48543–48549
- Morosinotto, T., Breton, J., Bassi, R. and Croce, R. (2003) The nature of a chlorophyll ligand in Lhca proteins determines the far red fluorescence emission typical of Photosystem I. *J. Biol. Chem.* **278**, 49223–49229
- Morosinotto, T., Castelletti, S., Breton, J., Bassi, R. and Croce, R. (2002) Mutation analysis of Lhca1 antenna complex: low energy absorption forms originate from pigment–pigment interactions. *J. Biol. Chem.* **277**, 36253–36261
- Morosinotto, T., Mozzo, M., Bassi, R. and Croce, R. (2005) Pigment–pigment interactions in Lhca4 antenna complex of higher plants Photosystem I. *J. Biol. Chem.* **280**, 20612–20619
- Mozzo, M., Morosinotto, T., Bassi, R. and Croce, R. (2006) Probing the structure of Lhca3 by mutation analysis. *Biochim. Biophys. Acta* **1757**, 1607–1613
- Croce, R., Chojnicka, A., Morosinotto, T., Ihalainen, J. A., van Mourik, F., Dekker, J.P., Bassi, R. and van Grondelle, R. (2007) The low-energy forms of Photosystem I light-harvesting complexes: spectroscopic properties and pigment–pigment interaction characteristics. *Biophys. J.* **93**, 2418–2428
- Ihalainen, J.A., Croce, R., Morosinotto, T., van Stokkum, I.H.M., Bassi, R., Dekker, J.P.X. and van Grondelle, R. (2005) Excitation decay pathways of Lhca proteins: a time-resolved fluorescence study. *J. Phys. Chem. B* **109**, 21150–21158
- Ihalainen, J. A., Ratsep, M., Jensen, P.E., Scheller, H. V., Croce, R., Bassi, R., Korppi-Tommola, J.E.I. and Freiberg, A. (2003) Red spectral forms of chlorophylls in green plant PSI: a site-selective and high-pressure spectroscopy study. *J. Phys. Chem. B* **107**, 9086–9093
- Romero, E., Mozzo, M., van Stokkum, I.H.M., Dekker, J.P., van Grondelle, R. and Croce, R. (2009) The origin of the low-energy form of Photosystem I light-harvesting complex Lhca4: mixing of the lowest exciton with a charge-transfer state. *Biophys. J.* **96**, L35–L37
- Ganeteg, U., Kulheim, C., Andersson, J. and Jansson, S. (2004) Is each light-harvesting complex protein important for plant fitness? *Plant Physiol.* **134**, 502–509
- Ganeteg, U., Strand, A., Gustafsson, P. and Jansson, S. (2001) The properties of the chlorophyll a/b-binding proteins Lhca2 and Lhca3 studied *in vivo* using antisense inhibition. *Plant Physiol.* **127**, 150–158
- Wientjes, E., Oostergetel, G.T., Jansson, S., Boekema, E. J. and Croce, R. (2009) The role of Lhca complexes in the supramolecular organization of higher plant Photosystem I. *J. Biol. Chem.* **284**, 7803–7810
- Croce, R., Canino, G., Ros, F. and Bassi, R. (2002) Chromophore organization in the higher-plant photosystem II antenna protein CP26. *Biochemistry* **41**, 7334–7343
- Weber, G. and Teale, F.W.J. (1957) Determination of the absolute quantum yield of fluorescent solutions. *Trans. Faraday Soc.* **53**, 646–655
- Klimmek, F., Ganeteg, U., Ihalainen, J.A., van Roon, H., Jensen, P.E., Scheller, H.V., Dekker, J.P. and Jansson, S. (2005) Structure of the higher plant light harvesting complex I: *in vivo* characterization and structural interdependence of the Lhca proteins. *Biochemistry* **44**, 3065–3073
- Mozzo, M., Passarini, F., Bassi, R., van Amerongen, H. and Croce, R. (2008) Photoprotection in higher plants: the putative quenching site is conserved in all outer light-harvesting complexes of Photosystem II. *Biochim. Biophys. Acta* **1777**, 1263–1267
- Horton, P., Ruban, A.V. and Walters, R.G. (1996) Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655–684
- Palacios, M.A., de Weerd, F.L., Ihalainen, J.A., van Grondelle, R. and van Amerongen, H. (2002) Superradiance and exciton (de)localization in light-harvesting complex II from green plants? *J. Phys. Chem. B* **106**, 5782–5787
- van Amerongen, H., Valkunas, L. and van Grondelle, R. (2000) *Photosynthetic Excitons*, World Scientific Publishing, Singapore

- 
- 50 Carbonera, D., Agostini, G., Morosinotto, T. and Bassi, R. (2005) Quenching of chlorophyll triplet states by carotenoids in reconstituted Lhca4 subunit of peripheral light-harvesting complex of Photosystem I. *Biochemistry* **44**, 8337–8346
- 51 Garab, G., Cseh, Z., Kovacs, L., Rajagopal, S., Varkonyi, Z., Wentworth, M., Mustardy, L., Der, A., Ruban, A.V., Papp, E. et al. (2002) Light-induced trimer to monomer transition in the main light-harvesting antenna complex of plants: thermo-optic mechanism. *Biochemistry* **41**, 15121–15129
- 52 Croce, R., Morosinotto, T. and Bassi, R. (2006) LHCl: the antenna complex of Photosystem I in plants and green algae. In *Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase* (Golbeck, J. H., ed.), pp. 119–137, Kluwer Academic Publisher, Dordrecht
- 53 Morosinotto, T., Ballottari, M., Klimmek, F., Jansson, S. and Bassi, R. (2005) The association of the antenna system to Photosystem I in higher plants. *J. Biol. Chem.* **280**, 31050–31058
- 54 Croce, R., Mozzo, M., Morosinotto, T., Romeo, A., Hiennerwadel, R. and Bassi, R. (2007) Singlet and triplet state transitions of carotenoids in the antenna complexes of higher-plant Photosystem I. *Biochemistry* **46**, 3846–3855
- 55 Rajagopal, S., Joly, D., Gauthier, A., Beauregard, M. and Carpentier, R. (2005) Protective effect of active oxygen scavengers on protein degradation and photochemical function in photosystem I submembrane fractions during light stress. *FEBS J.* **272**, 892–902
- 56 Wei, J., Yu, H., Li, L.B., Kuang, T.Y., Wang, J.S., Gong, Y.D. and Zhao, N.M. (2001) The photodamage process of pigments and proteins of PSI complexes from *Spinacia oleracea* L. *Chin. Sci. Bull.* **46**, 1812–1816
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## SUPPLEMENTARY ONLINE DATA

# The light-harvesting complexes of higher-plant Photosystem I: Lhca1/4 and Lhca2/3 form two red-emitting heterodimers

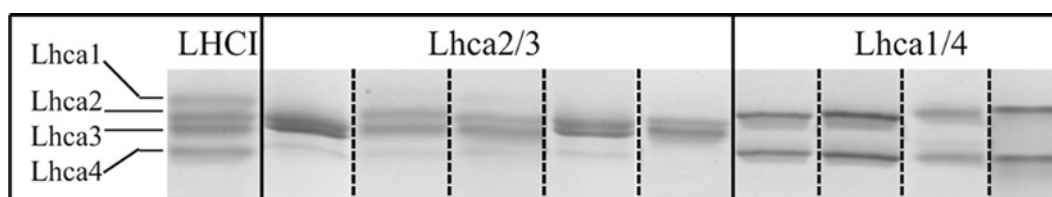
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**Table S1 Quantification of Coomassie Blue bound to Lhca polypeptides in the five preparations of Lhca2/3 presented in Supplementary Figure S1**

The amount of Coomassie Blue bound was determined by integrating the absorbance of each Lhca band; values compared were normalized to absorbance of Lhca2. Contamination of Lhca1/4 was determined by the absorbance of Lhca4 in the Lhca2/3 dimers compared with that of LHCI.

Sample	LHCI	Lhca2/3	Lhca2/3	Lhca2/3	Lhca2/3	Lhca2/3
Lhca1	0.80	0.05	0.06	0.04	0.07	0.00
Lhca2	1	1	1	1	1	1
Lhca3	1.28	1.40	1.33	1.54	1.25	1.90
Lhca4	1.06	0.05	0.08	0.07	0.09	0.01
Lhca1/4 contaminant	50 %	4.6 %	6.9 %	6.3 %	7.8 %	0.90 %



**Figure S1 SDS/PAGE analysis of dimeric Lhca complexes, obtained after solubilization of PSI-WT (LHCI), PSI-ΔLhca1 (Lhca2/3) and PSI-ΔLhca2 (Lhca1/4)**

Five different purifications of Lhca2/3 dimer and four of Lhca1/4 dimer are shown.

## Composition of dimeric Lhca complexes

All samples used in this work were analysed by SDS/PAGE (Supplementary Figure S1). The dimers, obtained by solubilizing PSI from Δa2 plants, were composed of Lhca1 and Lhca4. The dimeric fraction obtained after solubilization of PSI-Δa1 consisted mostly of Lhca2 and Lhca3; however, Lhca1 and Lhca4 were also present. Densitometric analysis of the gel (Supplementary Table S1) showed that the amount of Lhca1/4 was  $5.3 \pm 2.7$  %.

To evaluate the Lhca2/Lhca3 ratio in the dimer obtained after PSI-Δa1 solubilization, the amount of Coomassie Blue bound to the two polypeptides in the gel was compared. The staining was  $1.48 \pm 0.26$ -fold higher for Lhca3 than for Lhca2. Ballottari et al. [1] found that the affinity of Coomassie Blue binding is  $\sim 1.4$ -fold higher for Lhca3 than for Lhca2. This indicates that Lhca2 and Lhca3 are present in equal amounts, in agreement with a heterodimeric Lhca2/3 complex.

## Absorption properties of the red forms

In order to obtain more details about the red forms, the  $Q_y$  region of the absorption spectra was described as a sum of Gaussians.

A problem often encountered in this kind of analysis is that, owing to a lack of spectral structure in the red tail, more than one combination of Gaussians can describe the data. However, for the Lhca1/4 dimer, the position of the maximum of the red-most form can be discerned by second-derivative analysis ( $\sim 706$  nm). Furthermore, only one form should absorb above 705 nm as was shown in a site-selected and anisotropy fluorescence study on LHCI [2]. The Gaussian fit meeting this requirement is presented in Supplementary Figure S2(A). The red-most form shows its maximum at 706 nm and a FWHM (full width at half maximum) of 25 nm. In the absorption spectrum of the Lhca2/3 dimer, no clear maximum of the red-most form was observed by second-derivative analysis, giving rise to a larger uncertainty of the fit. The best result was obtained with the maximum of the red-most form at 707 nm with an FWHM of 25 nm (Figure S2B). The red-most Gaussian band represents 8.5 % and 8.9 % of the total oscillator strength in the  $Q_y$  region (630–750 nm) for Lhca1/4 and Lhca2/3 respectively.

## Reconstituted compared with native complexes

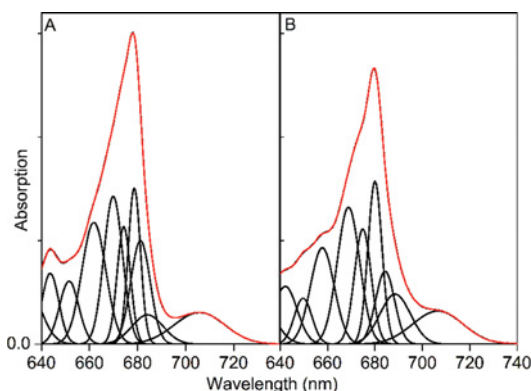
Owing to the difficulties in purification of the individual native Lhca antennas, a large part of our Lhca knowledge stems from the

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**Table S2** Pigment-binding properties of Lhca complexes

Cars in reconstituted Lhca, taken from [4,5]. Abbreviations used: n, native; nd, not detected; r, reconstituted.

	Chl <i>a/b</i>	Chl/Car	Violaxanthin	Lutein	$\beta$ -Car	Total Chls
rLhca1	4.0	3.3	1.0	1.8	nd	10
rLhca2	1.9	5.0	0.5	1.5	nd	10
rLhca3	6.0	3.5	0.7	1.6	0.5	10
rLhca4	2.4	4.8	0.4	1.7	nd	10
rLhca1/4	3.0	4.0	1.6	2.4	0.8	20
rLhca1+rLhca4	3.1	3.9	1.4	3.5	nd	20
rLhca2+rLhca3	3.1	4.1	1.2	3.1	0.5	20
nLhca1/4	3.7	4.8	1.3	2.7	1.0	24
nLhca2/3	3.7	4.7	1.0	2.5	1.6	24

**Figure S2** Gaussian analysis

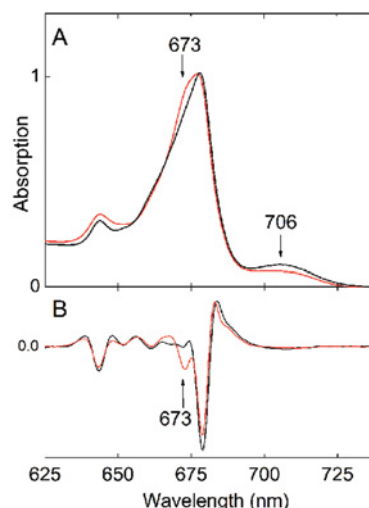
The  $Q_y$  region of the LT absorption spectra (red) of Lhca1/4 (A) and Lhca2/3 (B) described by Gaussian bands (black). Note that the fitting is only meant to extract the characteristics of the red bands, using the restrictions as explained in the supplementary text.

study of *in vitro* reconstituted complexes. However, on the basis of the analysis of a PSI preparation depleted in the individual complexes, it has been suggested that the native complexes could have different properties [3]. To check this suggestion, we directly compared the properties of the reconstituted complexes with those of the native antennas.

### Pigment composition

In Supplementary Table S2, the pigment composition of the native dimers is compared with the sum of the pigments in the reconstituted monomers [4]. On the basis of the properties of the monomers, identical Chl *a/b* and Chl/Car ratios are expected for the two dimers. This is indeed the case. The difference in the absolute value of the Chl *a/b* ratio (3.7 compared with 3.1) can be due to the absence of some Chls in the reconstituted complexes or to too low a Chl *a/b* ratio used in the reconstitution. Indeed, it has been suggested that the reconstituted complexes only co-ordinate ten Chl molecules [5], whereas in the crystal structure 13–14 Chls are associated with each complex [6]. We have tentatively normalized the number of Chls per native dimer to 24, taking into account that some of the Chls observed in the structure are located at the periphery of the complexes, and do not seem to belong to a specific protein. They are probably stabilized by protein–protein interaction, and are thus most likely to be lost during purification.

Although neoxanthin was present in the pigment mixture used for the reconstitution, the rLhcas (reconstituted Lhcas) do not co-ordinate this Car, thus indicating that the Car binding of the

**Figure S3** Absorption spectra at 77 K of native and reconstituted Lhca1/4

Absorption (A) and second derivative thereof (B) for native (black) and reconstituted (red) Lhca1/4. Absorption spectra are normalized to the area.

reconstituted complexes is specific. The amount of violaxanthin is very similar in the sum of the monomers when compared with the native dimer. Lutein is in both cases the most abundant Car, but in the native dimers the amount is smaller than in the sum of the monomers, whereas the opposite is true for  $\beta$ -carotene. The data thus suggest that  $\sim 1$  lutein is replaced by a  $\beta$ -carotene in both native dimers, as compared with the monomers. Interestingly, the reconstituted Lhca1/4 dimer was also shown to be able to co-ordinate  $\beta$ -carotene, although the two monomers were not [5]. This suggests that dimerization stabilizes the co-ordination of  $\beta$ -carotene, or that the binding of  $\beta$ -carotene is required for dimerization.

In conclusion, the comparison of the pigment composition of native and reconstituted complexes show very high similarity; the differences could be associated with the effect of the dimerization.

### Lhca1/4

In Supplementary Figure S3, the  $Q_y$  region of the LT absorption spectra of native and reconstituted Lhca1/4 dimers are compared. In general there is good agreement between the two spectra. However, rLhca1/4 shows less absorption at 706 nm and more around 673 nm. The second-derivative spectrum demonstrates that the band at 673 nm is only present in rLhca1/4. It has been suggested that Chl 603 and Chl 609, which are responsible for the red forms when they are in excitonic interaction, absorb at around 675 nm in the absence of such interaction [7]. Therefore it can be proposed that a fraction of the rLhca1/4 is lacking the strong excitonic Chl *a* interaction. However, although the oscillator strength of the red-most form is smaller in the reconstituted dimer as compared with the native one, the other properties of the band (maximum, FWHM) are similar in the two preparations (Supplementary Table S3 and [8]).

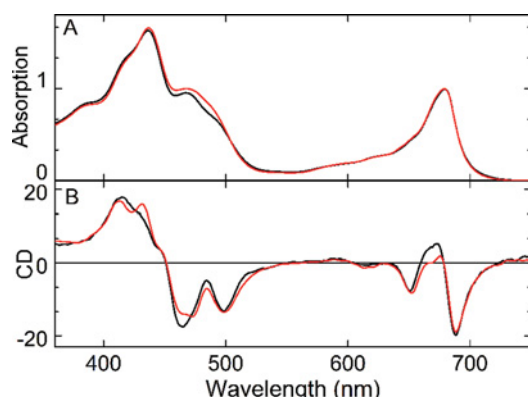
### Lhca2 and Lhca3

Unfortunately, it has not been possible so far to obtain a reconstituted Lhca2/3 dimer [9]. This is probably due to the fact that this dimer is far less stable than the Lhca1/4 dimer, as the present data also indicate, and therefore cannot endure the quite

**Table S3 Spectroscopic properties of the red forms**

Em, emission; hom, homogeneous; inh, inhomogeneous; tot, total.

	Absorbance (nm)	Area (%)	Em (nm)	Sv (cm <sup>-1</sup> )	FWHM <sub>tot</sub> (cm <sup>-1</sup> )	FWHM <sub>hom</sub> (cm <sup>-1</sup> )	FWHM <sub>inh</sub> (cm <sup>-1</sup> ) <sub>..</sub>
Lhca1/4	706	8.5	731.5	247	502	383	325
Lhca2/3	707	8.9	728.5	209	501	352	357

**Figure S4 Native and recombinant Lhca2 and Lhca3**

Absorption (A) and CD (B) spectra of a mixture of native Lhca2 and Lhca3 (black), obtained by light-induced monomerization of the Lhca2/3 dimer, and the sum of rLhca2 and rLhca3 spectra (red). Spectra are normalized to the Chl content.

harsh detergent treatment during the reconstitution procedure. Moreover, it has been demonstrated, in the case of the Lhca1/4 dimer, that dimerization induces changes in the spectroscopic properties of some of the chromophores [5], making it difficult to compare these properties for monomers and dimers. However, high-light treatment induced the monomerization of Lhca2/3 dimer without addition of detergent, giving the opportunity to compare the complexes in their monomeric aggregation state. In Supplementary Figure S4, the absorption and CD spectra of the monomeric Lhca2 and Lhca3 fractions, as obtained after light treatment of the native Lhca2/3 dimer (Figure 7 of the main text), are compared with the sum of the spectra of the reconstituted Lhca2 and Lhca3 complexes. It should be noted that the normalized CD (Figure 8 of the main text) and absorption (results not shown) spectra of the dimeric Lhca2/3 complex before and after light-treatment were very similar. Therefore the spectra of the monomeric complexes are also not expected to be strongly affected by the light. The striking similarity of the spectra shows that the pigment organization in the reconstituted complexes is virtually identical with that of the native ones.

### Spectroscopic properties of the red forms

Supplementary Table S3 shows the main properties of the red forms of the two dimers obtained from the steady-state measurements. The properties are very similar, and apart from the relative area, also comparable with those found for the refolded complexes [8].

Reported are: absorption wavelength, the relative contribution to the absorption on cm<sup>-1</sup> scale in the Q<sub>y</sub> region, fluorescence emission wavelength, optical reorganization energy (Sv) obtained from the Stokes shift (=2Sv), the FWHM<sub>tot</sub> of the absorption band described with a Gaussian shape,  $FWHM_{hom}^2 = 7.7SvT$  [10] and  $FWHM_{tot}^2 = FWHM_{hom}^2 + FWHM_{inh}^2$ .

### REFERENCES

- Ballottari, M., Govoni, C., Caffarri, S. and Morosinotto, T. (2004) Stoichiometry of LHCI antenna polypeptides and characterization of gap and linker pigments in higher plants Photosystem I. *Eur. J. Biochem.* **271**, 4659–4665
- Ihalainen, J.A., Gobets, B., Sznee, K., Brazzoli, M., Croce, R., Bassi, R., van Grondelle, R., Korppi-Tommola, J.E.I. and Dekker, J.P. (2000) Evidence for two spectroscopically different dimers of light-harvesting complex I from green plants. *Biochemistry* **39**, 8625–8631
- Klimmek, F., Ganeteg, U., Ihalainen, J.A., van Roon, H., Jensen, P.E., Scheller, H.V., Dekker, J.P. and Jansson, S. (2005) Structure of the higher plant light harvesting complex I: *in vivo* characterization and structural interdependence of the Lhca proteins. *Biochemistry* **44**, 3065–3073
- Croce, R., Mozzo, M., Morosinotto, T., Romeo, A., Hienrwadel, R. and Bassi, R. (2007) Singlet and triplet state transitions of carotenoids in the antenna complexes of higher-plant Photosystem I. *Biochemistry* **46**, 3846–3855
- Croce, R., Morosinotto, T., Castelletti, S., Breton, J. and Bassi, R. (2002) The Lhca antenna complexes of higher plants Photosystem I. *Biochim. Biophys. Acta* **1556**, 29–40
- Amunts, A., Drory, O. and Nelson, N. (2007) The structure of a plant Photosystem I supercomplex at 3.4 Å resolution. *Nature* **447**, 58–63
- Morosinotto, T., Breton, J., Bassi, R. and Croce, R. (2003) The nature of a chlorophyll ligand in Lhca proteins determines the far red fluorescence emission typical of Photosystem I. *J. Biol. Chem.* **278**, 49223–49229
- Croce, R., Chojnicka, A., Morosinotto, T., Ihalainen, J. A., van Mourik, F., Dekker, J.P., Bassi, R. and van Grondelle, R. (2007) The low-energy forms of Photosystem I light-harvesting complexes: spectroscopic properties and pigment–pigment interaction characteristics. *Biophys. J.* **93**, 2418–2428
- Schmid, V.H.R., Potthast, S., Wiener, M., Bergauer, V., Paulsen, H. and Storf, S. (2002) Pigment binding of Photosystem I light-harvesting proteins. *J. Biol. Chem.* **277**, 37307–37314
- Zucchelli, G., Garlaschi, F.M. and Jennings, R.C. (1996) Thermal broadening analysis of the light harvesting complex II absorption spectrum. *Biochemistry* **35**, 16247–16254